

In the Claims:

1. (Currently Amended) A method for isolating antigenic peptides in femtomolar amounts of about 16 to 320 femtomoles, which method comprises:
(a) providing complexes of MHC class II_peptide receptors with antigenic peptides isolated from a mammalian organism in an amount of 0.1 to 5 μ g by sequestering the complexes with immunoprecipitation, wherein the complexes are coupled to beads;
(b) washing the sequestered complexes; and
(c) eluting the associated antigenic peptides from the peptide receptors, wherein the elution is carried out in a single step.
wherein the femtomolar amount is about 16 to 320 femtomoles.
2. (Previously Amended) The method according to claim 1, further comprising isolating the complexes of MHC class II_peptide receptors with antigenic peptides in an amount of 0.1 to 5 μ g from cells isolated from a mammalian organism.
3. (Previously Amended) The method according to claim 2, wherein the complexes of MHC class II_peptide receptors with antigenic peptides are isolated from the cells with a method comprising solubilization of the cells with a detergent.
4. (Original) The method according to claim 2, wherein the cells isolated from a mammalian organism are dendritic cells.
5. (Previously Amended) The method according to claim 1, wherein (b) further comprises washing the sequestered complexes with water in an ultrafiltration tube before step (c).
6. (Previously Amended) The method according to claim 1, wherein the antigenic peptides are eluted from the MHC class II peptide receptors using 0.1% diluted acid

wherein the 0.1% diluted acid is selected from the group consisting of diluted acetonitrile (0.1%), diluted acetic acid (0.1%) and diluted trifluoro acetic acid (0.1%).

7. (Original) The method according to claim 1, wherein the isolated antigenic peptides are fractionated, sequenced and identified.

8. (Original) The method according to claim 7, wherein the isolated antigenic peptides are fractionated, sequenced and identified by methods comprising liquid chromatography and mass spectrometry.

9. (Original) The method according to claim 1, wherein the antigenic peptides are naturally-processed antigenic peptides or non-naturally processed antigenic peptides administered to the organism.

10. (Previously Amended) The method according to claim 1, wherein the peptide receptors comprise MHC II molecules.

11. (Previously Amended) The method according to claim 2, wherein the cells are cells expressing peptide receptors belonging to the group comprising MHC II molecules.

12. (Original) The method according to claim 1, wherein the mammalian organism is a human organism.

13. (Currently Amended) A method for isolating antigenic peptides in femtomolar amounts of about 16 to 320 femtomoles, which method comprises:

(a) providing complexes of MHC class II peptide receptors with antigenic peptides isolated from cells, tissue or body fluid of a mammalian organism in an amount of 0.1 to 5 μ g by sequestering the complexes with immunoprecipitation, wherein the complexes are coupled to beads;

(b) washing the sequestered complexes with water in an ultrafiltration tube;

(c) eluting the associated antigenic peptides from the peptide receptors at 37°C with 0.1% diluted trifluoro acetic acid; and
(d) sequencing and identifying the isolated peptides by liquid chromatography and mass spectrometry
~~wherein the femtomolar amount is about 16 to 320 femtomoles.~~

14. (Currently Amended) A method for isolating antigenic peptides in femtomolar amounts of about 16 to 320 femtomoles, which method comprises:

- (a) providing MHC class II expressing cells in a number providing 0.1 to 5 µg MHC molecules;
 - (b) contacting the cells of (a) with a source of potential antigen;
 - (c) isolating MHC class II molecule-antigenic peptide complexes from the cells by sequestering the complexes with immunoprecipitation, wherein the complexes are coupled to beads;
 - (d) washing the sequestered complexes; and
 - (e) eluting the associated peptides from the MHC class II molecules
- ~~wherein the femtomolar amount is about 16 to 320 femtomoles.~~

15. (Original) The method according to claim 14, wherein the MHC expressing cells are MHC I expressing cells.

16. (Cancelled)

17. (Original) The method according to claim ~~16~~ 14, wherein the MHC II expressing cells are dendritic cells.

18. (Original) The method according to claim 17, wherein the dendritic cells are exposed to a potential source of antigen as immature dendritic cells at the same time as they are induced to mature to dendritic cells.

19. (Original) The method according to claim 14, wherein the source of potential antigen belongs to the group comprising tumor cells, tumor cell lines, pathogens, viral, bacterial and parasitic antigens, autoantigens, body fluids e.g. serum, synovial fluid, ascites.

20. (Previously Amended) The method according to claim 14, wherein the complexes of MHC class II peptide receptors with antigenic peptides are isolated from the cells with methods comprising solubilization of the cells with a detergent and sequestration of the complexes of peptide receptors with antigenic peptides by immunoprecipitation or immunoaffinity chromatography.

21. (Previously Amended) The method according to claim 14, wherein the sequestered complexes of MHC class II peptide receptors with antigenic peptides are washed with water in an ultrafiltration tube before eluting the peptides.

22. (Previously Amended) The method according to claim 14, wherein the antigenic peptides are eluted from the MHC class II peptide receptors using 0.1% diluted acid wherein the 0.1% diluted acid is selected from the group consisting of diluted acetonitrile (0.1%), diluted acetic acid (0.1%) and diluted trifluoro acetic acid (0.1%).

23. (Original) The method according to claim 14, wherein the isolated antigenic peptides are fractionated, sequenced and identified.

24. (Original) The method according to claim 23, wherein the isolated antigenic peptides are fractionated, sequenced and identified by methods comprising liquid chromatography and mass spectrometry.

25. (Original) The method according to claim 23, wherein the antigenic peptides derived from the source of potential antigen are identified by comparing the peptides identified from cells which have been contacted with a source of potential antigen with those

which have been identified from cells which have not been contacted with a source of potential antigen.

26. (Original) The method according to claim 14, wherein the antigenic peptides are naturally-processed antigenic peptides.

27. (Currently Amended) A method for isolating antigenic peptides in femtomolar amounts of about 16 to 320 femtomoles, which method comprises

- (a) providing immature dendritic cells in a number providing 0.1 to 5 μ g MHC II molecules;
- (b) contacting the cells of (a) with a source of potential antigen and inducing maturation of dendritic cells by adding TNF α ;
- (c) isolating MHC II molecule-antigenic peptide complexes from the cells with methods comprising solubilization of the cells with the detergent TX-100 and sequestration of the complexes of MHC II molecules with antigenic peptides by immunoprecipitation;
- (d) washing the sequestered complexes of MHC II molecules with antigenic peptides with water in an ultrafiltration tube;
- (e) eluting the associated antigenic peptides from the MHC II molecules at 37°C with 0.1% diluted trifluoro acetic acid; and
- (f) sequencing and identifying the isolated peptides by liquid chromatography and mass spectrometry;

~~wherein the femtomolar amount is about 16 to 320 femtomoles.~~

28. (Withdrawn) A method for controlling the quality of a vaccine comprising:

- (a) providing MHC expressing cells in a number providing 0.1 to 5 μ g MHC molecules;
- (b) contacting the cells of (a) with a source of potential antigen;
- (c) isolating MHC molecule-antigenic peptide complexes from the cells; and

(d) eluting the associated peptides from the MHC molecules, wherein the number of tumor antigenic peptides bound to the MHC molecule is indicative of the quality of the vaccine.

29. (Withdrawn) A method for monitoring the stage of a disease comprising:

- (a) providing complexes of peptide receptors with antigenic peptides isolated from a mammalian organism in an amount of 0.1 to 5 μ g;
- (b) eluting the associated antigenic peptides from the peptide receptors; and
- (c) correlating the antigenic peptide with the stage or phase of the disease.

30. (Withdrawn) The method according to claim 29 wherein the disease is an autoimmune disease.

31. (Withdrawn) A method for controlling the efficacy of a therapeutic treatment comprising:

- (a) providing complexes of peptide receptors with antigenic peptides isolated from a mammalian organism in an amount of 0.1 to 5 μ g;
 - (b) eluting the associated antigenic peptides from the peptide receptors to monitor a pre-treatment antigenic peptide level;
 - (c) administering the therapeutic treatment to the mammalian organism;
 - (d) repeating steps (a) and (b) to obtain a post-treatment antigenic peptide level;
- and
- (e) correlating changes in peptide levels as indicia of the efficacy of the therapeutic treatment.

32. (Withdrawn) A pharmaceutical composition comprising the antigenic peptides isolated according to the method of claim 1 and a pharmaceutically acceptable carrier or diluent.

33. (Withdrawn) A pharmaceutical composition comprising the antigenic peptides isolated according to the method of claim 14 and a pharmaceutically acceptable carrier or diluent.